Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism

CHRISTIAN MEYER,¹ JEAN DOSTOU,¹ VEENA NADKARNI,¹ AND JOHN GERICH¹,²

Departments of ¹Medicine and ²Physiology and Pharmacology,
University of Rochester School of Medicine, Rochester, New York 14642

Meyer, Christian, Jean Dostou, Veena Nadkarni, and John Gerich. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. Am. J. Physiol. 275 (Renal Physiol. 44): F915–F921, 1998.—To determine the effect of physiological hyperinsulinemia on renal and hepatic substrate metabolism, we assessed systemic and renal glucose release and uptake, systemic and renal gluconeogenesis from glutamine, and certain aspects of systemic and renal glucose release. Both hepatic glucose release (HGR) and renal glucose release (RGR) increased from 1.61 ± 0.2 to 2.18 ± 0.25 mmol·kg⁻¹·min⁻¹ (P < 0.001), respectively. Renal glucose uptake (RGU) increased from 1.61 ± 0.19 to 2.18 ± 0.25 mmol·kg⁻¹·min⁻¹ (P = 0.029) but accounted for only ~5% of systemic glucose disposal (40.6 ± 4.3 mmol·kg⁻¹·min⁻¹). Both systemic and renal FFA clearance increased approximately fourfold (P < 0.001 for both). Nevertheless, renal FFA uptake decreased (P = 0.024) and was inversely correlated with RGU (r = −0.582, P = 0.011). Finally, insulin increased systemic glutamine release (P = 0.007), uptake (P < 0.005), and clearance (P < 0.001) but left renal glutamine uptake and release unaffected (P > 0.4 for both).

Liver; gluconeogenesis; free fatty acids

IT IS NOW WELL ESTABLISHED that, in postabsorptive humans, both liver and kidney release glucose into the circulation (26, 37). The liver is responsible for 70–80% of overall systemic glucose release under these conditions (5, 26, 37). However, because virtually all of the glucose released by the kidney is due to gluconeogenesis (46), the contributions of the liver and kidney to overall gluconeogenesis are roughly comparable (37).

Both hepatic glucose release (HGR) and renal glucose release have been shown to be influenced by a variety of hormones under in vitro conditions (11, 35). Insulin suppresses these processes in both organs, whereas other hormones, such as glucagon, epinephrine, vaso-

pressin, and parathyroid hormone, can stimulate them to varying degrees. Studies in normal volunteers, using the euglycemic clamp technique, have demonstrated complete suppression of systemic glucose release with physiological hyperinsulinemia (49). These results imply that, in humans, both renal glucose release and HGR are under physiological control by insulin. However, it is unclear to what extent and with what relative sensitivity insulin acts on these organs.

Certain observations suggest that there may be differences between hepatic and renal glucose release. Much of the glucose release by the liver is the result of glycogenolysis, a process that appears to be more sensitive to insulin than gluconeogenesis (6), whereas glucose release by the kidney is almost exclusively due to gluconeogenesis (46). Acidosis stimulates renal gluconeogenesis while suppressing hepatic gluconeogenesis (46). Although gluconeogenesis in both organs is stimulated by free fatty acids (FFA) (21, 44), liver and kidney differ in their use of gluconeogenic precursors, e.g., alanine is almost exclusively used by the liver (2) and glutamine is predominantly used by the kidney (27).

Moreover, epinephrine appears to augment hepatic gluconeogenesis indirectly by increasing precursor availability (34), whereas it probably acts on the kidney through a direct mechanism (35). Finally, although insulin does not increase splanchnic (or, presumably, hepatic) glucose uptake under euglycemic conditions (10), infusion of insulin into a renal artery has been shown to increase the glucose uptake of the infused kidney (5), suggesting insulin's direct renal effect. These observations are of particular interest in view of recent studies providing evidence that insulin may predominantly suppress HGR via indirect peripheral effects (1, 24).

The present studies were therefore undertaken to assess the relative effects of physiological hyperinsulinemia in suppressing hepatic and renal glucose release and gluconeogenesis from glutamine. In addition, we also assessed the effects of physiological hyperinsulinemia on renal and systemic glucose, glutamine, and FFA disposal.

METHODS

Subjects. We obtained informed written consent from nine normal volunteers (4 men and 5 women) after the protocol had been approved by the University of Rochester Institutional Review Board. The subjects were 31 ± 3 yr of age (means ± SE), weighed 75 ± 5 kg (body mass index of 24.9 ± 2.1 kg/m²), had normal glucose-tolerance tests according to World Health Organization criteria, and had no family history of diabetes mellitus. For 3 days before the study, all subjects were kept on a weight-maintaining diet containing at least 200 g carbohydrate and had abstained from alcohol.
Some of the baseline data of six subjects were included in a previous publication (26).

Protocol. Subjects were admitted to the University of Rochester General Clinical Research Center between 6:00 and 7:00 PM of the evening before the experiments. They consumed a standard meal (10 kcal/kg: 50% carbohydrate, 35% fat, and 15% protein) between 6:30 and 8:00 PM and were fasted overnight until experiments were completed.

At approximately 5:30 AM, an anteubital vein was cannulated and primed; continuous infusions of [6-3H]glucose (~30 µCi, ~0.3 µCi/min; Amersham International, UK) and of [U-14C]glutamine (~20 µCi, ~0.2 µCi/min; Amersham International) were started. At approximately 8:00 AM, an infusion of p-aminohippuric acid (12 mg/min) was started to determine renal blood flow (RBF). Between 8:00 and 9:00 AM, a renal vein was catheterized under fluoroscopy. At ~9:00 AM, an infusion of [9,10-3H]palmitate (~2 µCi/min; Amersham International) was initiated and a dorsal hand vein was cannulated and kept in a thermostated Plexiglas box at 65°C for sampling arterialized venous blood. Beginning ~1 h later, after allowing ~4 h for isotopic steady state to be achieved, three blood samples were collected from the dorsal hand vein and the renal vein simultaneously at 30-min intervals (~60, ~30, and ~0 min) to determine glucose, glutamine, glutamate, alanine, lactate, glycerol, FFA, insulin, glucagon, and p-aminohippuric acid concentrations and to assess [3H]- and [14C]glucose, [14C]glutamine, and [3H]palmitate specific activities. At 0 min, a continuous infusion of insulin (0.6 µU · kg⁻¹ · min⁻¹) was started via the anteubital infusion line. Subsequently, blood glucose concentrations were maintained at ~5 mM throughout the study, using the glucose-damp technique and a 20% glucose infusion enriched with [6-3H]glucose to minimize changes in plasma glucose specific activity (13, 49). Arterialized venous blood glucose was measured every 5 min. Blood was collected, as described, at 40-min intervals for the first 2 h (40, 80, and 120 min) and then at 30-min intervals for the last 2 h of the study (150, 180, 210, and 240 min).

Analytical procedures. Whole blood glucose was immediately determined in triplicate with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma glutamine, glutamate, alanine, and FFA concentrations, as well as [3H]- and [14C]glucose and [14C]glutamine specific activities, were determined by high-performance liquid chromatography (17, 28, 30). Lactate and glycerol concentrations were measured by microfluorometric methods. Insulin C-peptide and glucacon concentrations were determined by standard radioimmunoassays (26).

Calculations. Systemic (total) release and removal of glucose, glutamine, and palmitate from the circulation were determined with steady-state equations under basal conditions (47) and, subsequently, during the euglycemic clamp with the non-steady-state equations of DeBodo et al. (9), which were modified for glucose as described by Finegood et al. (13). The proportion of systemic glucose release in the steady state due to whole body glutamine gluconeogenesis was calculated as (arterial [14C]glucose SA/arterial [14C]glutamine SA) × 100/1.2, where SA is specific activity. Total glutamine gluconeogenesis was calculated as the proportion of glucose release due to glutamine multiplied by glucose release. During the non-steady-state condition (i.e., during insulin infusion), whole body glutamine gluconeogenesis was calculated using the equation of Chiasson et al. (7).

Renal plasma flow (RPF) and RBF were determined by the p-aminohippuric acid-clearance technique (3). Fractional extraction (FX) of glucose across the kidney was calculated as (arterial [6-3H]glucose concentration – renal vein [6-3H]glucose concentration)/(arterial [6-3H]glucose concentration).

Renal glucose uptake (RGU) was calculated as RBF × arterial glucose concentration × FX. Renal glucose net balance (NB) was calculated as RBF × (arterial glucose concentration – renal vein glucose concentration). Renal glucose release was calculated as RGU – NB. Analogous equations were used for [14C]glutamine and [3H]palmitate, except that RPF was used. Renal NB of other substrates was calculated as described for glucose, except that RPF was used for alanine and glycerol. HGR was calculated as the difference between systemic glucose release and renal glucose release.

Renal gluconeogenesis from glutamine was calculated as RBF × renal vein [14C]glucose concentration – (1 – FX) × (arterial [14C]glucose concentration)/1.2 × (renal vein [14C]glutamine SA), where FX is the renal fractional extraction of [3H]glucose.

Hepatic gluconeogenesis was calculated as the difference between systemic and renal glutamine gluconeogenesis. Assumptions and limitations of the combined NB and the isotopic approach for determining glucose release and glutamine gluconeogenesis by liver and kidney have been previously discussed in detail (26, 30).

Statistical analysis. Unless otherwise stated, data are expressed as means ± SE. Paired two-tailed Student’s t-tests were used to compare the means of three baseline determinations with mean data obtained from the last two time points of the euglycemic clamp. P < 0.05 was considered statistically significant.

RESULTS

Arterial insulin, glucose, C-peptide, and glucagon concentrations. During infusion of insulin, plasma insulin concentrations averaged ~220 µM, and plasma glucose concentrations were maintained at 4.97 ± 0.03 mM (coefficient of variation of 4.1 ± 0.4%) (Fig. 1). Plasma C-peptide and glucagon concentrations both decreased by 20% (P = 0.014 and P < 0.001, respectively).

Systemic, renal, and hepatic glucose metabolism. Infusion of insulin decreased overall systemic glucose release by ~50% from 11.0 ± 0.4 to 5.37 ± 0.51 µmol · kg⁻¹ · min⁻¹ during the last hour (P < 0.001) (Fig. 2). Renal glucose release decreased from 2.29 ± 0.45 to 0.90 ± 0.20 µmol · kg⁻¹ · min⁻¹ (P < 0.002) but was nearly maximally suppressed within the first 80 min. HGR decreased less abruptly from 8.71 ± 0.4 to 4.47 ± 0.46 µmol · kg⁻¹ · min⁻¹ (P < 0.001). Although the absolute decrement in glucose release was greater for liver than for kidney, insulin reduced HGR to a lesser extent than renal glucose release in terms of percent suppression (47.3 ± 6.0 vs. 60.7 ± 4.3%, P = 0.027).

Systemic glucose disposal increased nearly fourfold from 11.0 ± 0.4 to 40.6 ± 4.3 µmol · kg⁻¹ · min⁻¹ (P < 0.001) (data not shown). RBF (Table 1) increased by ~15% by the end of the infusion of insulin (P < 0.001) and RGU increased by ~30% (P = 0.029), although renal glucose FX was not significantly increased (P = 0.506).

Systemic and renal glutamine metabolism. Arterial glutamine concentrations (Fig. 3) decreased by ~15% during the insulin infusion (P = 0.001), despite the fact that the systemic release of glutamine (Fig. 4) in-
creased (P = 0.007). This was probably due to the fact that, during the early part of the insulin infusion, systemic glutamine disposal increased to a slightly greater extent than its release. Systemic glutamine clearance increased by ∼25% from 10.0 ± 0.8 to 12.8 ± 0.6 ml·kg⁻¹·min⁻¹ (P < 0.001). Renal glutamine NB, FX, uptake, and release were unchanged (Table 1).

Systemic, renal, and hepatic glutamine gluconeogenesis. Before infusion of insulin, renal (0.52 ± 0.07 µmol·kg⁻¹·min⁻¹) and hepatic (0.35 ± 0.03 µmol·kg⁻¹·min⁻¹) glutamine gluconeogenesis accounted for 60.6 ± 2.8 and 39.4 ± 2.8% of systemic glutamine gluconeogenesis, respectively (0.87 ± 0.08 µmol·kg⁻¹·min⁻¹) (Fig. 4). Infusion of insulin decreased systemic glutamine gluconeogenesis by ∼50% to 0.41 ± 0.03 µmol·kg⁻¹·min⁻¹, P < 0.001. Renal and hepatic glutamine gluconeogenesis decreased to 0.14 ± 0.03 (P < 0.001) and 0.27 ± 0.03 µmol·kg⁻¹·min⁻¹ (P < 0.002), respectively, during the last hour. As was the case for glucose release, insulin suppressed renal glutamine gluconeogenesis to a greater extent than hepatic glutamine gluconeogenesis (71.6 ± 5.2 vs. 24.7 ± 4.9%, P < 0.001). Moreover, the time course of changes in renal and hepatic gluconeogenesis differed; renal glutamine gluconeogenesis decreased within 40 min and was maximally suppressed by 120 min, whereas hepatic glutamine gluconeogenesis increased during the initial 80 min before finally decreasing below baseline values.

Arterial concentrations and renal NBs of glutamate, alanine, lactate, and glycerol. Arterial glutamate concentrations decreased by ∼20% during the last hour of the insulin infusion (P < 0.001) despite an increase of the renal net release of glutamate. Arterial alanine,...
concentrations and renal NB remained essentially unchanged. In contrast, arterial lactate concentrations and renal lactate net uptake both increased (P < 0.01). Arterial glycerol concentrations decreased by 75% (P < 0.001). This could wholly account for the reduced renal glycerol uptake because renal glycerol FX remained unchanged (51.5 ± 6.8 vs. 55.3 ± 1.7% basal, P = 0.617).

Systemic and renal FFA metabolism. Arterial FFA concentrations (Fig. 4) decreased by ~80% (P < 0.001) during the last hour of the insulin infusion. Systemic FFA release (Fig. 5) decreased by ~40% (P < 0.002). Despite the fact that systemic FFA disposal decreased (P < 0.002), systemic FFA clearance increased approximately fourfold (P < 0.001). Renal FFA FX increased (P < 0.001) and was positively correlated with systemic FFA clearance (r = 0.889, P < 0.001). Because there was no significant renal FFA release, renal FFA NB closely approximated renal FFA uptake (Table 1). This was inversely correlated with RGU (r = -0.582, P = 0.011).

**DISCUSSION**

In the present studies, infusion of insulin in normal postabsorptive volunteers, which produced physiological hyperinsulinemia (~200 pM) comparable to values observed postprandially (29), suppressed overall kidney and liver glucose release as well as their production of glucose from glutamine. Moreover, the percent suppression of renal glucose release and glutamine gluconeogenesis was greater than that of HGR and glutamine gluconeogenesis, indicating that the kidney was more sensitive to insulin. Physiologically, this would make sense because the arterial insulin concentrations to which the kidney is exposed are considerably lower than the portal venous insulin levels to which the liver is exposed.
It is of interest that both renal glucose release and renal glutamine gluconeogenesis followed similar time courses, being maximally suppressed by ~120 min. This is consistent with the fact that renal glucose release is largely due to gluconeogenesis. In contrast, hepatic glutamine gluconeogenesis and HGR did not parallel one another and took a longer time to reach maximal suppression. HGR decreased progressively over the initial 120 min, whereas hepatic glutamine gluconeogenesis actually increased during the initial 80 min.

An explanation for this seemingly paradoxical increase in hepatic glutamine gluconeogenesis is unclear but could involve hepatic autoregulation (16), wherein a decrease in hepatic glycogenolysis causes an increase in hepatic gluconeogenesis analogous to the situation in which an increase in hepatic gluconeogenesis causes a reduction in hepatic glycogenolysis. Another possibility is the insulin stimulation of hepatic amino acid transport (36). The hepatic amino acid A-transporter system, which is partly responsible for glutamine uptake, is stimulated by insulin, whereas it is not in the kidney (36). Consistent with this, we found no change in renal glutamine FX and uptake, whereas there was a significant increase in systemic glutamine uptake and clearance, which could have involved the liver. Still another possibility is that some of the glutamine gluconeogenesis actually represented gluconeogenesis from [14C]glutamine in the kidney or other tissues. Finally, it is also possible that our assumption of equivalent changes in hepatic and renal Krebs cycle carbon exchange may not be valid. If Krebs cycle carbon exchange decreased in the liver, then this would have led to an overestimation of hepatic glutamine gluconeogenesis.

Regarding the cause of the initially increased hepatic glutamine gluconeogenesis, if the changes in hepatic glutamine gluconeogenesis reflected hepatic gluconeogenesis in general, then this would imply that the observed early suppression of HGR was entirely attributable to suppression of glycogenolysis. This would be consistent with observations of Chiasson et al. (6) demonstrating that, in dogs, hepatic glycogenolysis is more sensitive than gluconeogenesis to insulin suppression.

The demonstration in the present study, that renal glucose release is suppressed by physiological insulin concentrations, indicates that insulin is a physiological regulator of renal glucose release. One might therefore expect that renal glucose release might be increased in insulin-resistant conditions such as type II diabetes (25). Indeed, increased renal glucose release has been demonstrated to occur in insulin-resistant animals such as alloxan or streptozotocin diabetic rats (19, 22, 39) and glucocorticoid-treated rats (39, 43).

Insulin suppression of renal glucose release should largely reflect suppression of renal gluconeogenesis. Renal glycogen stores are generally negligible, and cells that could store glycogen lack glucose-6-phosphatase and cannot release glucose via glycogenolysis (46). Glutamine is the predominant amino acid used for gluconeogenesis by the kidney (38). Because its uptake by the kidney was unaltered during infusion of insulin and because renal uptake of lactate (another major renal gluconeogenic precursor) increased during infusion of insulin, it seems likely that insulin suppressed renal gluconeogenesis by altering intrarenal disposal of gluconeogenic precursors rather than by altering their transport into the kidney. This could have occurred through inhibition of gluconeogenic enzymes and/or by stimulation of alternate pathways for disposal of potential gluconeogenic precursors.

Our data cannot provide insight into the relative importance of these potential mechanisms. However, it is of note that the activity of several renal gluconeogenic enzymes is increased in insulin-deficient diabetic animals, implying that insulin can suppress the activity of those enzymes (23). Moreover, in present studies before insulin infusion, nearly all renal glutamine uptake could be accounted for by the conversion of glutamine to glucose. During insulin infusion, only about one-third of glutamine uptake could be accounted for by conversion to glucose. Some of the glutamine carbon might have been diverted to glutamate, the renal release of which was increased during infusion of insulin, and some of the glutamine could have been used as an oxidative fuel in place of FFA, the renal uptake of which was diminished.

Regarding glucose uptake, we found that insulin stimulated RGU to a moderate extent (~20%). Because whole body glucose uptake increased to a much greater extent (~400%), it would appear that the human kidney is not a major site of insulin-mediated glucose disposal. In the studies of Cersosimo et al. (5), in which insulin was infused locally into the renal artery of dogs without causing systemic hyperinsulinemia, glucose uptake by the infused kidney increased by ~75%. Because arterial insulin levels were probably increased to a greater extent in our studies, these differences could indicate that the RGU may be more sensitive to insulin in dogs than in humans.

In the present studies, renal glucose FX was not significantly altered by insulin. About 60% of the increase in RGU could be accounted for by an increase in RBF induced by insulin. A similar effect of insulin on RBF has been observed in previous studies (12, 31). Moreover, in the present studies, plasma FFA levels and renal FFA uptake decreased. There was a significant inverse correlation between renal FFA and glucose uptake, with ~35% of the variation in RGU being explained by the variation in renal fatty acid uptake \( r = -0.582, P = 0.011 \). These latter observations are consistent with the concept of a renal glucose-fatty acid cycle (26) and, taken together with the changes in RBF, suggest that infusion of insulin may increase RGU in a manner other than by directly stimulating renal glucose transport.

Although in the present study insulin reduced renal and systemic FFA uptake, both renal FX and systemic FFA clearance increased significantly. The latter is consistent with previous reports of an increase in systemic FFA clearance during hyperinsulinemic
euglycemic clamp experiments in normal postabsorptive humans (14, 42). Insulin suppresses systemic FFA oxidation while stimulating overall FFA reesterification (4). One might expect a similar mechanism to be operative in the kidney, especially because the insulin-induced increase in RGU should reduce renal FFA oxidation and increase availability of glycerol-3-phosphate for reesterification of FFA. This would be consistent with findings of in vitro studies indicating that triglyceride synthesis is an important route of FFA disposal in the kidney (45).

The design of the present studies also permitted evaluation of the effects of insulin on systemic and renal glutamine metabolism, which have not been previously studied. Infusion of insulin increased systemic glutamine turnover (both uptake and release) without significantly altering renal FX, uptake, and release. Thus the systemic effects of insulin on glutamine metabolism are attributable to extrarenal tissues. Although the increase in systemic glutamine disposal might have been anticipated from the known effects of insulin to stimulate tissue-amino acid transport and protein synthesis (33, 40), it was surprising to also observe an increase in systemic glutamine release. The effect of insulin to suppress proteolysis (15, 40) would have been expected to reduce systemic glutamine release if this were the major factor influencing systemic glutamine release in the circulation.

An increase in the conversion of plasma glucose to glutamine, the so-called glucose-glutamine cycle (32), could provide a possible explanation for the increased systemic glutamine release during insulin infusion. Under postabsorptive conditions, ~12% of glutamine carbons entering the circulation originate from plasma glucose (32); this represents ~5% of systemic glucose disposal. In the present study, infusion of insulin increased systemic glucose disposal approximately fourfold. If a comparable proportion of the increased glucose disposal involved the conversion of glucose to glutamine, this would have increased systemic glutamine release by about ~0.7 mmol·kg⁻¹·min⁻¹, which is nearly threefold more than the observed increase in glutamine release (~0.5 mmol·kg⁻¹·min⁻¹).

Thus an increase in the glucose-glutamine cycle would have been able to offset a decrease in glutamine release due to suppression of proteolysis and would result in a net increase in glutamine release into the circulation. This postulate of an insulin-induced increase in the glucose-glutamine cycle seems plausible because increases in the Cori (glucose-lactate) cycle occur during euglycemic hyperinsulinemic clamp experiments (8, 41, 48). Physiologically, the operation of these cycles would permit anabolic actions of insulin (protein and glycogen formation) while sustaining appropriate glucose release into the circulation via provision of gluconeogenic precursors. It is of note, however, that in the present study there was increased net renal uptake of lactate and no change in renal glutamine release during the infusion of insulin. Thus it seems unlikely that the kidney was involved in increases in these cycles.

Finally, some comment is warranted regarding Krebs cycle carbon exchange. It is well established that carbon exchange in the Krebs cycle can cause an underestimation of isotopic determination of systemic gluconeogenesis as performed in the present studies (18). We have assumed that such underestimation would be comparable for liver and kidney, as suggested from in vitro studies (20), and thus would not disproportionately affect hepatic glutamine gluconeogenesis calculated as the difference between systemic and renal glutamine gluconeogenesis. However, there is little support for this assumption. It could be argued that during infusion of insulin, when glucose flux was increased, Krebs cycle carbon exchange would not have been reduced. This might not have been operative in kidney, where glucose uptake was unaltered, but may have occurred in liver, where glucose uptake might have increased. If so, this would have resulted in a relative overestimate of hepatic glutamine gluconeogenesis.

In summary, the present studies indicate that, in humans, physiological concentrations of insulin exert numerous actions on renal substrate homeostasis and extrarenal glutamine metabolism. Suppression of glutamine gluconeogenesis is more sensitive to insulin in the kidney than in the liver, whereas stimulation of RGU plays a minor role in insulin-stimulated glucose disposal and may in fact represent an indirect action of insulin mediated by changes in RBF and renal FFA uptake rather than a direct effect on renal glucose transport.

We thank the staff of the General Clinical Research Center for excellent technical help and Mary Little for superb editorial support. The present work was supported in part by National Institutes of Health Grants 5M01-RR-00044 and DK-20411.

Address for reprint requests: J. E. Gerich, Univ. of Rochester School of Medicine, 601 Elmwood Ave., Box MED/CRC, Rochester, NY 14642

Received 24 April 1998; accepted in final form 20 August 1998.

REFERENCES


